

(12) **UK Patent Application** (19) **GB** (11) **2 151 599 A**

(43) Application published 24 Jul 1985

(21) Application No 8428179

(22) Date of filing 7 Nov 1984

(30) Priority data

(31) 8330124

(32) 11 Nov 1983

(33) GB

(71) Applicant

United Kingdom Atomic Energy Authority (United Kingdom),
11 Charles II Street, London SW1Y 4QP

(72) Inventor

Dr Alan Rosevear

(74) Agent and/or Address for Service

Clive Stephen Bennett,
Patents Branch, United Kingdom Energy Authority, 11
Charles II Street, London SW1Y 4QP

(51) INT CL⁴

B01J 20/02

(52) Domestic classification

C1A 302 308 421 424 M10 M12 N34 N4 VF1
U1S 1361 C1A

(56) Documents cited

GB 1503315

GB 1272287

GB 0923109

GB 1312438

GB 1002207

(58) Field of search

C1A

(54) **Composite materials having thiol groups**

(57) A composite material having thiol groups comprises a rigid support material.

The composite material may comprise a deformable gel (eg agarose) having thiol groups retained within the pore structure of a porous rigid support material (e.g. Kieselghur).

The particles of composite material are used to prepare a radioactive gold isotope from a mercury "parent" isotope.

GB 2 151 599 A

SPECIFICATION

Improvements in or relating to materials

- 5 The present invention relates to materials and particularly to composite materials.

According to one aspect of the present invention there is provided a composite material having thiol groups comprising a rigid support material having thiol groups.

- 10 Preferably the composite material having thiol groups comprises a material having thiol groups retained within the pore structure of a porous rigid support material.

- 15 Preferably the material having thiol groups is, or is part of, a deformable gel and the composite material comprises the deformable gel retained within the pore structure of a porous rigid support material.

- 20 By "deformable gel" we mean a gel which itself is a non-rigid substrate (e.g. a xerogel). Such deformable gels include organic polymeric materials. An example of such organic polymeric materials is a polyol (e.g. a synthetic polymer gel of polyvinyl alcohol or a polysaccharide gel (such as agarose gel or a cellulose gel)).

- As used in this Specification "thiol groups" embraces precursors convertible thereto by methods known in the art, and derivatives thereof obtainable by methods known in the art.

- 30 In one embodiment thiol groups may be attached to a deformable gel (e.g. a polyol) by a short methylene chain. For example thiopropyl groups may be attached to a polyol.

- Preferably the porous rigid support material is in the form of discrete porous particles. For example the porous rigid support material may be in the form of discrete porous particles having interconnected porosity such as may be prepared by a method as claimed in any one of claims 1 to 9 or claim 15 of British Patent Specification No 1421531

- 45 (UKAEA) (to which US Patent 3943072 corresponds). Examples of such particles are discrete porous particles of inorganic oxides such as TiO_2 and Al_2O_3 and discrete porous particles of natural earths such as Celite (Trade Mark) and Kieselguhr).

- The term "aeogel" has been used in the art to describe a rigid, preformed matrix containing pores and this term and the term "xerogel" are discussed in "An Introduction to Permeation Chromatography" by R. Epton and C. Hollway issued by Koch-Light Laboratories Limited.

- According to another aspect of the present invention there is provided a method for preparing a composite material of a deformable gel having thiol groups retained within the pore structure of a porous rigid support material comprising introducing a precursor for the gel into the pore structure of a porous rigid support material and treating the precursor

to form and retain the deformable gel in the pore structure.

- 70 It will be appreciated that the majority of the gel will be present in the internal pore structure of the porous rigid support material, but also it should be noted that some gel may be formed on the surface of the support material.

- The present invention also provides a method for preparing a composite material of a deformable gel having thiol groups retained within the pore structure of a porous rigid support material, which method includes the steps of treating an inactive deformable gel (as hereinafter defined) retained within the pore structure of a porous rigid support material to provide the inactive deformable gel with thiol groups.

- By "inactive deformable gel" we mean a deformable gel having no useful thiol groups. The inactive deformable gel can be treated to provide it with thiol groups by modifying the inactive gel or by adding thiol groups thereto.

- Where the thiol groups are part of a deformable gel, it will be appreciated that other constituents of the deformable gel in the finished composite material should not, in general, have properties which interfere with the thiol groups.

- 95 An example of a composite material in accordance with the present invention is discrete particles of Kieselguhr containing an agarose gel which has been treated such that the agarose gel has thiol groups. Composite materials in accordance with the present invention may find application in the recovery of chemical species from fluid; for example in one application a composite material in accordance with the present invention may be used in the recovery of heavy metals (e.g. Hg, U, Pb and Cd) from aqueous solutions (e.g. for detoxification, purification, recovery purposes or analysis).

- A composite material in accordance with the present invention may also find application in the production of radioactive isotopes (e.g. gold isotopes). Thus, a mercury "parent" isotope may be held on a composite material having thiol groups and the gold "daughter" isotope eluted by a suitable reagent. Reference may be made to copending Application No. (Agent Reference) 13454 M1R (UKAEA) of even date in relation to the production of a gold isotope in this manner.

- 120 Other examples of applications of composite materials in accordance with the present invention are protein recovery or immobilisation (e.g. by oxidative coupling with thiol groups).

- In view of the foregoing statements in this specification it will be appreciated that where a deformable gel is used the porous rigid support material provides a rigid "skeleton" having dimensional stability as a support for the non-rigid deformable gel. Thus deformable gels which have, or can be treated to have,

thiol groups, but which are difficult or inconvenient to handle because of their non-rigid nature (e.g. hydrogels which will undergo dimensional changes when subjected to pressures normally found in column operation (e.g. up to ~3 atmospheres) and deform to cause increase in back pressure), are incorporated into a composite material of the present invention which, due to the rigidity imparted by the porous rigid support material "skeleton", can be handled and used more easily.

Thus where the composite material comprises, for example, porous discrete particles with a deformable gel retained therein the composite materials can be loaded into, and used, conveniently in column systems.

It has been found that if deformable gels having thiol groups are retained in accordance with the invention in discrete porous particles (for example porous particles of a natural earth such as Celite or Kieselguhr made by a method as claimed in our British Patent Specification No 1421531 hereinbefore mentioned) the composite materials comprising deformable gel, having thiol groups and rigid support materials possess desirable properties. Thus the particles of composite material tend to settle readily in aqueous media and can be used to form columns having good flow properties. Also the particles of composite material tend to be stable and not liable to release "fines".

The particles of composite material therefore may be, for example, introduced into columns and used to remove chemical species from solution.

Examples of porous rigid support materials suitable for use in forming composite materials in accordance with the present invention are disclosed in British Patent Specification No 1421531 hereinbefore mentioned. For example, using one type of the discrete porous particles disclosed therein discrete porous particles of composite material have been prepared comprising porous rigid particles of Kieselguhr having retained therein a deformable gel having thiol groups.

Discrete porous particles as disclosed in British Patent Specification No 1421531 and being of materials other than Kieselguhr may, of course, be used in accordance with the present invention.

A number of methods may be used to prepare an inactive deformable gel in the pore structure of the porous rigid support material prior to treating to provide the gel with thiol groups.

Thus according to the one embodiment of the present invention a precursor for an inactive deformable gel is introduced into the pore structure of the porous rigid support material in solution and the solution in the support material is subsequently treated with a precipitating agent to cause precipitation of a gel from the precursor solution. The gel can be

subsequently treated to provide thiol groups.

According to a further embodiment of the present invention a precursor for an inactive deformable gel is introduced into the pore structure of the porous rigid support material in solution and the solution in the support material is subsequently cooled to cause gelation and formation of a gel from the precursor solution.

The gel can be subsequently treated to provide thiol groups.

An example of gelation by cooling is the cooling of an agarose solution to <35°C to give a stable hydrogel.

Examples of precipitation reactions which may be used in carrying out the method of the present invention are:

(i) the regeneration of celluloses or cellulosic ion exchangers from solution of the corresponding xanthates (e.g. by decomposition of the xanthates of cellulose, DEAE-cellulose or CMC-cellulose by aqueous mineral acids); and
(ii) precipitation of acidic polysaccharides with acid or calcium salts.

In another embodiment of a method in accordance with the present invention a precursor can be introduced into the pore structure of the porous rigid support material and subsequently polymerized to form a polymer gel in the pore structure (e.g. acrylic acid derivatives may be introduced to the pore structure and subsequently polymerized to give gels of polymers and co-polymers of the derivatives) (e.g. acrylamide or an acryloyl cysteine). The polymer gel may be treated as necessary to provide thiol groups.

In a further embodiment of the invention cross-linking of the precursor can be used to form a gel.

The cross-linking may be carried out with a chemical cross-linking chemical agent by diffusing the agent into the pore structure in order to react with the precursor. It is very desirable to carry out the cross-linking under conditions such that significant quantities of the precursor cannot diffuse out of the porous rigid support material whilst the cross-linking agent is diffusing into the porous rigid support material. This can be achieved by temporarily retaining the precursor in the support material (e.g. by precipitation) to hold it available in the pore structure thereof for cross-linking and subsequently treating the temporarily retained precursor to cross-link it. It will be appreciated that by "temporarily retaining the precursor" we mean that the precursor is "localised" in the pore structure of the support substantially to prevent it diffusing out as the cross-linking agent diffuses in. Where the precursor has been introduced to the porous material in aqueous solution precipitation can be achieved, for example, by contacting the aqueous precursor solution with a water miscible organic solvent (e.g. acetone) capable of removing water from the aqueous solution

thereby to precipitate precursor in the pore structure.

For example DEAE-dextran, polysaccharides and neutral polyols can be precipitated from aqueous solution using acetone as the water miscible organic solvent and cross-linked by use of epichlorohydrin. Examples of substances which can be used to produce a deformable gel in a composite material in accordance with the present invention by precipitation and cross-linking are dextran sulphate, CMC-cellulose, acrylamide, agarose and P.V. alcohol.

Cross-linking agents for use in accordance with the present invention can be, for example, epichlorohydrin, bis epoxides, or dihalo compounds for polyols and, for example, dialdehydes for proteins.

Where the precursor, precipitation mechanism and cross-linking agent are such that the cross-linking of the precursor to form the gel is slow in comparison with the rate of precipitation, the precursor and cross-linking agent can be introduced into the pore structure of the porous rigid support material together in one solution (i.e. because precipitation will be effected before cross-linking occurs).

In one particular embodiment an agarose gel may be formed within the pores of a porous rigid support material (e.g. fabricated Kieselguhr particles), a portion of the hydroxyl group may be subsequently derivatised (e.g. with epichlorohydrin), sodium thiosulphate may then be used to give S-alkyl thiosulphate agarose and dithiothreitol may then be used to give thiopropyl agarose in the pores of the porous rigid support material.

In another embodiment a polysaccharide gel can be formed in the pores of a porous rigid support material and thiol groups attached by cyanogen bromide activation of the gel follows by attachment of a multifunctional compound containing a thiol ligand (e.g. glutathione).

In a further embodiment a synthetic thiol polymer may be synthesised directly in the pore of a porous rigid support material.

For example N-acryloyl derivatives of thiol compounds such as cysteine can be polymerised or co-polymerised with other acrylates by addition polymerisation to give a gel having thiol groups.

By way of further example thiol groups may be directly substituted onto a polymer such as polyvinyl alcohol by reaction with hydroiodic acid and thiourea and subsequent hydrolysis. Thiolated derivatives can also be prepared from amino-styrene polymers via a diazo compound to give a xanthate and hence a free thiol substituent.

In a further embodiment of the present invention thiol groups may be attached directly to the surface of an inorganic support material using thiolated derivatives of substituted triethoxysilanes.

As hereinbefore disclosed the majority of the gel may be arranged to be present in the internal pore structure of the porous rigid support material.

Thus in accordance with the invention it is possible to produce a composite material in which there is the minimum of deformable gel outside of the internal pore structure of the porous rigid support material. Thus, where the porous rigid support material is in the form of discrete porous particles there is a minimum of deformable gel formed between the particles, and substantially all of the deformable gel formed is retained by the particles with the majority of the deformable gel being in the internal pore structure thereof so that the resulting composite material is in the form of discrete particles such as to aid, *inter alia*, handling, column packing and column operation.

Loosely adhering deformable gel may be removed from the particles of composite material by washing and, if necessary, mechanical means, e.g. sieving.

To assist in maximising the amount of the deformable gel, or inactive deformable gel, retained in the pore structure of the porous rigid support material where, in accordance with an embodiment of the invention a solution of precursor is contacted with the porous rigid support material to introduce precursor into the pore structure, we prefer that the volume of the solution of precursor contacted with the support material (e.g. by soaking the support material in the solution) is approximately equal to the volume required to fill the pore structure. It will be appreciated that to minimise the amount of deformable gel, or inactive deformable gel, formed outside the pore structure the volume of the solution should not exceed the volume required to fill the pore structure.

It will be appreciated that in general the viscosity of the solution of the precursor should be such that it does not prevent uptake thereof by the support material (e.g. by capillary action).

Also it is preferred that the volume of any reagent solutions used to treat the precursor in the pores structure to form a gel is not substantially in excess of that required to immerse the porous rigid support material.

It will be appreciated that the present invention is not limited to composite material which can be used in aqueous solution and that composite materials can be prepared which may be used in non-aqueous systems.

It will be appreciated that the deformable gel and porous rigid support material should be substantially insoluble in fluid substances with which they may be contacted in use (e.g. solutions containing chemical compounds to be sorbed, feed solutions containing proteins, and eluting agent solutions).

The invention also provides a composite

material having thiol groups whenever prepared by a method in accordance with the invention.

The invention also provides a composite material having thiol groups obtainable by a method in accordance with the invention.

British Patent Specification No. 1,421,531 (UKAEA) discloses and claims, *inter alia*:

"A method for producing discrete porous particles for the selective retention of macromolecules from a fluid substance containing said macromolecules, said discrete porous particles having interconnected porosity throughout, which method includes the steps of preparing discrete green particles from a mixture containing solid particles of a finely divided, substantially insoluble, sorptive (as defined in the Specification), inorganic material and a fugitive additive in solution, said mixture being formed by mixing said solid particles of inorganic material with a fugitive additive and a solvent therefor, said fugitive additive being for subsequently providing a pore structure in the inorganic material and said inorganic material being substantially insoluble in the solvent for the fugitive additive, the preparation of the discrete green particles being such that, and the fugitive additive being such that, the fugitive additive is provided in solid form in the green particles, and heating the green particles to remove fugitive additive therefrom and cause sintering of inorganic material to give discrete porous particles, the fugitive additive and the amount thereof in the green particles being selected such that the discrete porous particles have an interconnected porosity throughout the discrete porous particles providing an extended surface area and the pore structure is such as will allow the macromolecules to permeate the discrete porous particles and be sorbed." and also claims discrete porous particles made by the method of British Patent No. 1,421,531.

British Patent Specification No. 1,574,414 discloses and claims, *inter alia*:

"A composite material comprising a plurality of discrete porous particles of a porous rigid support material having a deformable gel retained within the pore structure of the particles of porous rigid support material".

Discrete porous particles (for example those fabricated from a finely divided, substantially insoluble, sorptive inorganic material by a method as claimed in any one of claims 1 to 9 or claim 15 of British Patent No. 1,421,531) for use in accordance with the present invention preferably have a porosity of >20% and an interconnected porosity with pores $\geq 2000 \text{ \AA}$ such as to allow both deformable gel to occupy the pores.

"Celite" (Registered Trade Mark) as hereinbefore mentioned is a natural diatomaceous earth produced by Johns-Manville Corp.

It is believed that porous materials, having dimensional stability such as foam metal and

plastic foams can be used as porous rigid support materials in accordance with the present invention.

The invention will now be further described, by way of example only, as follows:

Example

Preparation of kieselguhr particles containing agarose having thiopropyl groups

Discrete porous particles of Kieselguhr (fabricated in accordance with BP 1,421,531 and substantially spherical and of 150–250 μ diameter) were filled with a 4% w/v gel of agarose (Litex) as follows:

Dry particles of Kieselguhr (50ml) were heated to 70°C in an oven and a hot solution of agarose (4%, 23 ml) was mixed therewith to produce an homogeneous particulate mixture. This mixture was allowed to cool for 30 minutes and the resulting particles were suspended in cold water and forced through a 300 μ sieve to break up any aggregates.

These discrete particles filled with agarose gel (50ml bed volume) were washed with distilled water and a 1M aqueous solution of sodium hydroxide (40 ml) was added and allowed to equilibrate in a reaction vessel. Epichlorohydrin (7.5 ml) was added dropwise (in a fume hood) with swirling to assist even distribution to give a suspension.

An air condensor was fitted to the reaction vessel and the suspension heated to 60°C by means of a water bath (in a fume hood) for 1.5 hr with occasional swirling. No oily phase was evident after approximately 0.5 hr.

The resulting reaction mixture was allowed to cool and the particulate material present was filtered out on a No. 1 glass sinter using a water pump.

The particulate material was washed with water (4 \times 50 ml aliquots) and 0.5 phosphate buffer (pH 6.3) (4 \times 50 ml aliquots). The particulate material was resuspended in this phosphate buffer (100 ml) and a solution of sodium thiosulphate added. The resulting suspension was occasionally swirled and left at room temperature for 6 hrs after which the particulate material was washed in water on a funnel and stored overnight in water.

The particulate material was washed in 0.1M sodium bicarbonate/1mM EDTA buffer and then suspended in 100ml of this buffer.

A fresh solution of dithiothreitol (0.5 g) in 1mM EDTA (15ml) was added and the resulting mixture swirled at room temperature. After 0.5 hr the supernatant was purple. The particulate material was washed with 0.1M sodium bicarbonate/1mM EDTA/1M NaCl (3 \times 50ml aliquots) and finally washed with 0.01M sodium acetate/1mM EDTA buffer (pH 4) (4 \times 50 ml aliquots).

The resulting particles of composite material were stored at 40°C in a sealed container in buffer (having a composition of the last wash) through which nitrogen had been bubbled for

0.5 hr.

The particles of composite material were found to be useful in the preparation of a radioactive gold isotope, by holding a mercury "parent" isotope on the particles of composite material and eluting the gold "daughter" isotope product by suitable reagents.

CLAIMS

1. A composite material having thiol groups comprising a rigid support material having thiol groups.
2. A composite material as claimed in Claim 1 wherein the composite material having thiol groups comprises a material having thiol groups retained within the pore structure of a porous rigid support material.
3. A composite material as claimed in Claim 2 wherein the material having thiol groups is, or is part of, a deformable gel and the composite material comprises the deformable gel retained within the pore structure of a porous rigid support material.
4. A composite material as claimed in Claim 2 or Claim 3 wherein the deformable gel includes an organic polymeric material.
5. A composite material as claimed in Claim 4 wherein the organic polymeric material is a polyol.
6. A composite material as claimed in Claim 1 wherein the deformable gel is a polyvinyl alcohol gel or a polysaccharide gel.
7. A composite material as claimed in Claim 6 wherein the gel is an agarose gel or a cellulose gel.
8. A composite material as claimed in any one of Claims 2 to 7 wherein thiol groups are attached to a deformable gel by a short methylene chain.
9. A composite material as claimed in any of Claims 1 to 7 wherein the porous rigid support material is in the form of discrete porous particles.
10. A composite material as claimed in Claim 9 wherein the porous rigid support material is in the form of discrete porous particles having interconnected porosity.
11. A composite material as claimed in any one of the preceding claims wherein the rigid support material comprises discrete porous particles of TiO_2 , of Al_2O_3 , or of a natural earth.
12. A method for preparing a composite material of a deformable gel having thiol groups retained within the pore structure of a porous rigid support material comprising introducing a precursor for the gel into the pore structure of a porous rigid support material and treating the precursor to form and retain the deformable gel in the pore structure.
13. A method as claimed in Claim 12 for preparing a composite material of a deformable gel having thiol groups retained within the pore structure of a porous rigid support material, which method includes the steps of

treating an inactive deformable gel (as hereinbefore defined) retained within the pore structure of a porous rigid support material to provide the inactive deformable gel with thiol groups.

14. A method as claimed in Claim 13 wherein a precursor for an inactive deformable gel is introduced into the pore structure of the porous rigid support material in solution, the solution in the support material is subsequently treated with a precipitating agent to cause precipitation of a gel from the precursor solution, and the gel is subsequently treated to provide thiol groups.
15. A method as claimed in Claim 14 wherein precipitation is effected by the regeneration of cellulose or cellulosic ion exchanger from a solution of the corresponding xanthate or by precipitation of acidic polysaccharides with acid or calcium salts.
16. A method as claimed in Claim 13 wherein a precursor for an inactive deformable gel is introduced into the pore structure of the porous rigid support material in solution, the solution in the support material is subsequently cooled to cause gelation and formation of a gel from the precursor solution, and the gel subsequently treated to provide thiol groups.
17. A method as claimed in claim 12 or 13 wherein a precursor is introduced into the pore structure of the porous rigid support material and subsequently polymerised to form a polymer gel in the pore structure.
18. A method as claimed in Claim 12 or 13 wherein cross-linking of a precursor is used to form a gel.
19. A method as claimed in Claim 12 or Claim 13 wherein a precursor for a gel is introduced to a porous support material, the precursor is temporarily retained on the support material by precipitation and subsequently treated to effect cross-linking of the precursor.
20. A method as claimed in Claim 19 wherein the precursor for a gel is a DEAE-dextran, a polysaccharide, or a neutral polyol.
21. A method as claimed in Claim 19 where the precursor for the gel is dextran sulphate, CMC-cellulose, acrylamide, agarose or polyvinyl alcohol.
22. A method as claimed in any one of Claims 19 to 21 wherein a cross-linking agent is used to effect cross-linking and said cross-linking agent comprises epichlorohydrin, a bis-epoxide, a dihalo compound or a dialdehyde.
23. A process as claimed in any one of Claims 13 to 22 wherein an agarose gel is formed within the pores of a porous rigid support material, hydroxyl groups are derivatised, sodium thiosulphate is used to give S-alkyl thiosulphate and dithiothreitol is used to give thiopropyl agarose in the pores of the support material.
24. A process as claimed in any one of

Claims 13 to 22 wherein a polysaccharide gel is formed in the pores of a porous rigid support material and thiol groups are attached by cyanogen bromide activation of the gel followed by attachment of a multifunctional compound containing a thiol ligand.

5
10 25. A process as claimed in Claim 12 wherein a synthetic thiol polymer is synthesised directly in the pores of a porous rigid support material.

26. A process as claimed in Claim 25 wherein N-acryloyl derivatives of thiol compounds are polymerised or co-polymerised with other acrylates by addition polymerisation to give a gel having thiol groups.

15
20 27. A process as claimed in any one of Claims 13 to 27 wherein thiol groups are directly substituted onto a polymer by reaction with hydrosulfuric acid and thiourea and subsequent hydrolysis.

28. A process for preparing a composite material having thiol groups wherein thiol groups are attached directly to the surface of an inorganic support material using a thiolated derivative of a substituted triethoxysilane.

25 29. A method for preparing a composite material substantially as hereinbefore described with reference to the Example.

30 30. A composite material having thiol groups whenever prepared by a method as claimed in any one of Claims 12 to 29.

31. A composite material having thiol groups obtainable by a method as claimed in any one of Claims 12 to 29.

35 32. A composite material substantially as hereinbefore described with reference to the Example.

Printed in the United Kingdom for
Her Majesty's Stationery Office, Dd 8818935, 1985, 4235
Published at The Patent Office, 25 Southampton Buildings,
London, WC2A 1AY, from which copies may be obtained.

THIS PAGE BLANK (USPTO)